Multicomponent Criteria for Predicting Carcinogenicity: Dataset of 30 NTP Chemicals

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This article is in response to the challenge issued to the scientific community by the National Toxicology Program to predict the carcinogenicity potential of 30 chemicals previously selected for long-term carcinogenicity testing. Utilizing the available toxicologic, genetic, and structural information on 30 chemicals previously selected for long-term carcinogenicity testing, we predict that 16 chemicals (53%) would induce some indication of carcinogenic activity in rodents; we further predict that 10 chemicals (33%) would be associated with weak or equivocal carcinogenic responses, and another 4 (13%) would give no indication of carcinogenicity. Our level of certainty is indicated for many of these predictions. Nonetheless, we believe that most instances of guessing whether a chemical would eventually induce cancer in experimental animals and hence represent a carcinogenic hazard to humans are fraught with considerable uncertainty: uncertainty that can only be relieved by long-term testing for carcinogenicity in animals or by conducting an epidemiologic investigation of exposed individuals or groups. We further believe that the day may come when our predictive acumen will be upgraded to such an extent that we might eventually obviate cancer testing. Until then, and in the best interests of public health, however, we urge long term testing of chemicals in animals be continued, at increased pace. — Environ Health Perspect 104(Suppl 5):1105–1112 (1996)

Key words: carcinogenicity, prediction criteria, NTP chemicals

Introduction

A formidable challenge among health professionals centers on the ability to predict, with reasonable accuracy, toxicologic and carcinogenic activities of exposure circumstances without actually having to conduct experimental research deemed necessary and essential to identify human health hazards. Humans and most other species are exposed to a plethora of both natural and synthetic chemicals in myriad, complex, and matrixal compositions (*1,2*). Unfortunately, most risk assessments are done on single entities that typically have little to do with real exposures. Most of these assessments come historically from experimental carcinogenicity studies and testing on single chemicals (*3*). Both now and in the future, more emphasis must be given to studying both simple and complex mixtures, as well as consumer, environmental (e.g., home), and occupational and workplace exposure circumstances (*4*).

In this article, we are responding to the challenge of the National Toxicology Program (NTP) to the scientific community to predict the carcinogenicity potential of 30 chemicals currently being tested by the NTP. Our predictions (read, "educated estimates or guesses") are based on available data on other chemicals and short term testing data on the 30 chemicals. Much of our educated estimating or guessing involved what we know already about other chemicals or metabolites that have been tested. These data include structure–activity relationships, physical and chemical properties, classes of agents (e.g., nitrosamines, aromatic amines, anthraquinones, and others known to be carcinogenic); mutagenicity and genetic toxicity; metabolic activation to reactive molecules (e.g., epoxides); mechanisms of carcinogenesis, and preneoplastic or neoplastic indicators identified in shorter term toxicity experiments. These criteria have been summarized, most notably by Weisburger (*5*), Fung et al. (*6*), and Woo et al. (*7*).

The newest entry into this prediction melange is mechanism (*8–12*); yet mechanisms of carcinogenesis, albeit exciting, remain virtually futuristic and must receive continuing study and verification before acceptance into the scientific, regulatory, and legislative complex (*13–15*). Unfortunately this has happened already.

We also include in this article the predictions of the National Cancer Institute (NCI) Chemical Selection Working Group (CSWG), which nominated 25 of the 30 chemicals for carcinogenicity testing. The CSWG has been in existence since the early 1970s. It is an interagency group of federal scientists that currently operates under the auspices of the Chemical and Physical Carcinogenesis Branch, Division of Cancer Biology, NCI. The CSWG serves as an evaluation committee in the NCI chemical selection process for nominating chemicals for carcinogenicity and other toxicity testing; reported to the NCI before 1978, and since then to the NTP. Because the CSWG's predictions were based on a much smaller database than that available to the current predictors, we thought it would be interesting to compare the success of the CSWG's predictive rate with that of the other participants in the NTP challenge exercise.

We surmise that one of the reasons for this challenge exercise is to determine how good the various predictive methodologies are and their potential for replacing the bioassay. Therefore, we thought it would be remiss not to emphasize that the bioassay is still the best way to ascertain whether a chemical is carcinogenic in rodents and that it would be unwise to decide against testing based on information from short-term studies. Accordingly, in the discussion section we provide examples of chemicals that were found to be carcinogenic in bioassays but for which there were no suggestions of carcinogenic activity in the short-term studies, and chemicals that were found to be noncarcinogenic but for which there were suggestions of carcinogenicity potential from the short-term studies.

Methods

In addition to the comments in the preceding paragraph, many computerized methods are currently used for predicting

This paper is part of the NIEHS Predictive-Toxicology Evaluation Project. Manuscript received 5 June 1996; manuscript accepted 8 August 1996.

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Abbreviations used: CSWG, Chemical Selection Working Group; NCI, National Cancer Institute; NTP, National Toxicology Program; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCE, trichloroethylene.

carcinogenic potential of agents not yet tested. In a previous NTP challenge exercise on 44 chemicals being tested, the most successful predictive rate was reported to be that of "human experts" (*16–18*). Not surprising, the ability of computer models to predict carcinogenicity can only be as good as the dataset on which each method was based. As we have learned and were prepared for in advance, it is exceedingly difficult to create machine/computer methods that incorporate all the various factors and biological modalities and idiosyncrasies that need to be considered in predicting carcinogenicity of chemicals (*4,14*).

So far, of the computer models available, the U.S. Environmental Protection Agency's Expert Oncologic System appears to come closest to bringing together the parameters used typically by a human expert.

Further, if one does concentrate on computer-prediction models, one should remain critical of results that may be contrary to established scientific thought; that is, computers are often wrong. Thus, it is clear that computer predictions should be scrutinized by human experts regarding both logical and scientific sense. Obviously, then, there is still a vital need for scientific intuition that is lacking in computerized models. With this in mind, and calling ourselves human experts, we attempted to utilize the totality-of-information approach to predict the potential carcinogenicity of 30 chemicals that are currently being tested or whose long-term studies were recently completed. Our predictions are based mainly on the criteria briefly mentioned above and described in more detail below, and on our scientific intuition—or naiveté—when there are insufficient or inadequate data available.

The criteria used to assign suspicion of carcinogenicity to a chemical included

- positive or suggestive results from previous experimental studies in animals or epidemiological studies
- close structural relationship to known carcinogens or chemical classes of carcinogens
- potential to act directly as an electrophilic agent or to be metabolized to an electrophilic species
- potential to act as a DNA intercalating agent
- potential to be metabolized to active free radical species
- positive results from mutagenicity studies (e.g., in *Salmonella typhimurium*).

Information on the absorption, distribution, metabolism and excretion of chemicals was also considered in the evaluation of the carcinogenic potential of the candidate chemicals. Thus, physical properties such as solubility in water and organic solvents; partition coefficients; and vapor pressure, which impact on the bioavailability of the chemicals were also given consideration.

For some chemicals, we elected to predict equivocal results because both toxic and detoxication mechanisms may be operative, and we were not able to estimate which would be the dominant mechanism.

Results

At the time of nominating and selecting chemicals for toxicologic characterization, including carcinogenesis, the CSWG typically rendered a guess—or prediction—for carcinogenic potential of the selected chemical. The CSWG's predictions were based on the criteria described above; however, at the time of doing this there was very little or no biologic or toxicologic information available on these chemicals. Table 1 lists the 30 chemicals and the CSWG's predictions: The CSWG did not evaluate 5 of the chemicals and therefore recorded no judgment on them; for the remaining 25, 16 were predicted to be carcinogenic (64%), 8 were considered unknown (32%), and one (xylenesulfonic acid, sodium) was considered to be noncarcinogenic (4%). Obviously this reflects the policy, which was prevalent at that time, of selecting and testing chemicals most likely to present carcinogenic risks to humans (*6,19*).

Nearly two decades later, on the basis of our intuitive sense and experience in evaluating chemicals for carcinogenic activity, and the availability of much more biologic and toxicologic information, we believe 16 of the 30 chemicals (53%) would be carcinogenic to one or more of the experimental groups configured in the typical two-species bioassay (*3,4,20*); 4 of the 30 chemicals (13%) would be noncarcinogenic, and 10 (33%) would be equivocal or weakly carcinogenic (Table 1). The three tabular columns contain the alphabetical listing of chemicals, with CAS registry numbers and the route of exposure; our predictions together with the rationale for this decision, as well as supplementary decision making comments; and the historic CSWG earlier predictions.

For several of these chemicals our placement into a particular category was more certain than for others. Some predictions were particularly difficult and represent real or significant guesses. In a few cases, disagreement among us remains; and these received consensus based on compromise and majority rules. For these few, we await the test results to learn who was the correct predictor.

We attempted initially to predict carcinogenic potential of these chemicals by individual species, strain, and gender: male rats, female rats, male mice, female mice. This proved to be unsatisfactory to us and was abandoned, for the most part, as being particularly fanciful. Thus, except where specified, our predictions are given on a chemical basis and not on each of the four individual experimental test units within that chemical bioassay. In certain instances we thought to follow various indicators (e.g., aliphatic solvents typically cause liver tumors in mice and perhaps kidney tumors in male rats) gleaned from previous bioassays, but even with these cases we decided this would be frivolous.

Discussion and Conclusions

In our view, except in rare cases these predictions, while being intellectually interesting and enjoyable, do not and likely will not replace eventual *in vivo* testing (*2,4,6, 14,19,21,22*). We offer here a few examples of chemicals that were tested although there was no way to anticipate the results. No one predicted that phthalates (di-2-ethylhexyl phthalate) would be carcinogenic in long-term exposure studies (*23–25*); however, the findings of carcinogenicity for the phthalates led to a large research effort directed toward mechanistic relevance of chemicals causing peroxisome proliferation. We were reproved for testing, among other chemicals, *d*-limonene; yet this oil-of-orange induced tumors of the kidneys in male rats (*26*), concomitantly associated with a testoster one-stimulated protein (α2μ globulin) that purportedly plays some mechanistic role in this lesion (*27–31*). Again these testing results—as well as findings for unleaded gasoline—stimulated much research into mechanisms. Also, so-called nongenotoxic carcinogens (*32*) identified in long-term bioassays have generated much speculation and hypotheses into mechanisms. Both di-2-ethylhexyl phthalate and *d*-limonene are nongenotoxic; hence other mechanisms are sought for their activity.

Much of this effort centers on the association or lack thereof of chemically induced toxic lesions leading to or progressing to benign and malignant tumors. Most large studies find little or no correlation or consistency between toxicity and carcinogenicity (*28,33,34*). Often empirical

Table 1. Authors' and NCI Chemical Selection Working Group's predictions.

Chemical CAS number Route of exposure	Authors' predictions; Rationale/Comments	NCI CSWG's Predictions
Anthraquinone 84-65-1 Feed	Noncarcinogenic Anthraquinone is metabolized to 2-hydroxyanthraquinone, which may be expected to be excreted via conjugation. Mutagenic; induced *in vivo* micronuclei. Although several anthraquinones were carcinogenic in NTP studies, these compounds had amino substituents and the observed carcinogenic effects may be attributed to the aromatic amine functionality rather than to the hydroquinone moiety. Hydroxyanthraquinones that are carcinogenic are substituted in the 1- or 8-position and may form a pyrene-type compound via hydrogen bonding of the hydroxy group and carbonyl function .	Carcinogenic
t-Butylhydroquinone 1948-33-0[a] Feed	Equivocal Induced chromosomal aberrations (CAs) and sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells. Hyperplasia observed in subchronic studies (male and female rats: hyperplasia of nasal respiratory epithelium; male and female rats: hyperplasia of skin and forestomach). Possibility of excretion via conjugation.	Did not consider
1-Chloro-2-propanol 127-00-4[a] Drinking water	Carcinogenic Mutagenic and clastogenic; negative *in vivo* micronucleus assay. Alkylating agent. Suggestive positive results from previous studies. Note: Drinking water studies are rarely positive.	Carcinogenic
Chloroprene 126-99-8[a] Inhalation	Carcinogenic Genotoxic. Structurally related to vinyl chloride and butadiene, known human and rodent carcinogens.	Carcinogenic
Cinnamaldehyde 104-55-2 Feed	Carcinogenic Weakly mutagenic. Alkylating agent; potential for forming iminium compound with DNA. Hyperplasia of the forestomach mucosa observed in both sexes of rats and mice in a microencapsulation subchronic study.	Carcinogenic
Citral 5392-40-5 Feed	Carcinogenic Nongenotoxic (SCEs in CHO). Potential alkylating agent; formation of iminium compound with DNA. Estrogenic activity. Sebaceous gland hyperplasia observed after topical application.	Carcinogenic
Cobalt sulfate heptahydrate 10026-24-1[a] Inhalation	Equivocal Weakly positive in SA. Previous studies indicated local sarcomas. Subchronic studies indicate proliferation, inflammatory and metaplastic changes in rats and mice. Essential micronutrient at physiological levels but may be carcinogenic at high levels.	Carcinogenic
Codeine 76-57-3[a] Feed	Noncarcinogenic Nongenotoxic (SCEs in CHO). Although there is potential for epoxide formation, and formation of a secondary amine which may be nitrosated, it is known that the main metabolic pathway for codeine is conjugation and excretion. Codeine and its metabolites are rapidly excreted within 24 hr. No nitrosated product was found.	Unknown suspicion
D&C Yellow No. 11 8003-22-3[a] Feed	Equivocal Positive results in mutagenicity and clastogenicity studies but did not induce *in vivo* micronuclei. Structurally related to quinoline, a rodent carcinogen.	Did not consider
Diethanolamine 11-42-2[a] Dermal	Carcinogenic Nongenotoxic. Potential for forming nitrosamine, namely, *N*-nitrosodiethylolamine, a liver and nasal carcinogen in rats.	Carcinogenic
1,2-Dihydro-2,2,4-trimethylquinoline 147-47-7[a] Dermal	Carcinogenic Increase in micronuclei seen in *in vivo* 90-day mouse study. *In vitro* SCE increase. Structurally related to quinoline, and suggestive positive results from earlier studies.	Carcinogenic

(Continued)

Table 1. (*Continued.*)

Chemical CAS number Route of exposure	Authors' predictions; Rationale/Comments	NCI CSWG's Predictions
Emodin 518-82-1 Feed	Carcinogenic Mutagenic, clastogenic and induced micronuclei. Emodin would be expected to form a glucuronide and subsequent facile excretion. However, there is some evidence for the tumor promoting carcinogenicity ability of hydroxyanthraquinones. 1- or 8-Hydroxyanthraquinones may form a pyrenetype structure via hydrogen bonding of the hydroxy and carbonyl functions.	Did not consider
Ethylbenzene 100-41-4[a] Inhalation	Equivocal Not mutagenic. Metabolized to α-methylbenzyl alcohol, which showed some evidence of carcinogenicity in male rats.	Did not consider
Ethylene glycol monobutyl ether 111-76-2[a] Inhalation	Noncarcinogenic Nongenotoxic. No structural alert.	Did not consider
Furfuryl alcohol 98-00-0[a] Inhalation	Carcinogenic Nongenotoxic. Induced SCEs in CHO cells. Structurally related to furan, which was carcinogenic in NTP studies. Carcinogenic effect expected to result from formation of epoxide.	Unknown suspicion
Gallium arsenide 1303-00-0 Inhalation[a]	Equivocal Nongenotoxic. Prediction is based on results of subchronic studies, for example, bone marrow hyperplasia in rats and pulmonary hyperplasia in mice.	Unknown suspicion
Isobutene 115-11-7[a] Inhalation	Carcinogenic Nongenotoxic. Potential for formation of epoxide. The epoxide of isobutene was mutagenic in *Salmonella* assay. Isobutene is a potential metabolite of methyl *t*-butyl ether, which is a rodent carcinogen.	Carcinogenic
Isobutyraldehyde 78-84-2[a] Inhalation	Carcinogenic Mutagenic and clastogenic. Potential alkylating agent–formation of iminium compound with DNA. It is possible that the length of the side chain may reduce the activity of the aldehyde function. Isobutyraldehyde was nominated to investigate this possibility.	Carcinogenic
Methyleugenol 93-15-2[a] Gavage	Carcinogenic Nongenotoxic (SCEs in CHO). Structurally related to safrole and estragole, known rodent carcinogens. Potential for formation of epoxide. Biliary hyperplasia observed in mice in subchronic studies. Eugenol was noncarcinogenic in NTP studies. In eugenol, the hydroxy substituents afforded a detoxifying mechanism via conjugation and ready excretion. In methyl eugenol, the hydroxyl groups are replaced by methoxy groups, which would block the detoxifying mechanism by the hydroxyl groups in eugenol.	Carcinogenic
Molybdenum trioxide 1313-27-5[a] Inhalation	Equivocal Nongenotoxic. Mutagenicity of ammonium molybdate. Essential micronutrient at physiological levels but may be carcinogenic at high levels.	Unknown suspicion
Nitromethane 75-52-5[a] Inhalation	Carcinogenic Nongenotoxic. Structurally related to tetranitromethane and 2-nitropropane, known rodent carcinogens. Degeneration of olfactory epithelium in subchronic studies in rats and mice. Potential for inducing cell proliferation. Potential activity via its tautomer, $CH_2{=}N(OH){=}O$.	Carcinogenic
Oxymetholone 434-07-1[a] Gavage	Carcinogenic Nongenotoxic. Promoter of liver carcinogenesis in rats. In subchronic studies, mammary gland hyperplasia was observed in female rats, and clitoral gland hyperplasia in female mice. Androgenic steroid; possible human carcinogen.	Carcinogenic

(Continued)

Table 1. (*Continued.*)

Chemical CAS number Route of exposure	Authors' predictions; Rationale/Comments	NCI CSWG's Predictions
Phenolphthalein 77-09-8[a] Feed	Carcinogenic Clastogenic and induced *in vivo* micronuclei. The lactone ring is susceptible to nucleophilic attack with subsequent formation of a resonance-stabilized electrophilic intermediate.	Carcinogenic
Primaclone 125-33-7[a] Feed	Carcinogenic Mutagenic. Potential for formation of a nitrosamine. Subchronic studies suggest potential for carcinogenic effect.	Carcinogenic
Pyridine 110-86-1[a] Drinking water	Equivocal Nongenotoxic. Metabolism studies suggest possibility of epoxide formation, similar to that observed in that of benzene.	Unknown suspicion
Scopolamine hydrobromide trihydrate 6533-68-2[a] Gavage	Equivocal Nongenotoxic. A suspicion of carcinogenicity may be based on the epoxide moiety and potential for formation of a nitrosamine; however, one might expect the epoxide moiety to be unreactive due to its hindered location. Appears to be readily absorbed and excreted ($t_{1/2}$ = 3 hr).	Carcinogenic
Sodium nitrite 7632-00-0 Drinking water	Equivocal or noncarcinogenic Mild squamous hyperplasia in forestomach of rats and mice observed in 90-day water study.	Unknown suspicion
Tetrahydrofuran 109-99-9[a] Inhalation	Equivocal Nongenotoxic. Epigenetic mechanism. Structurally related to dioxane. Little 90-day toxicity; liver in mice.	Unknown suspicion
Vanadium pentoxide 1314-62-1 Inhalation	Carcinogenic Nonmutagenic. In a 90-day study, hyperplasia was observed in respiratory epithelium of the nose in male and female rats and mice. Essential micronutrient at physiological levels, but may be carcinogenic at high levels.	Unknown suspicion
Xylenesulfonic acid, sodium salt 1300-72-7 [8] Dermal	Noncarcinogenic Nongenotoxic (did induce SCEs in CHO). An isomeric mixture of xylenes was noncarcinogenic in NTP studies. The sulfonic acid moiety is expected to exert a detoxifying effect.	Noncarcinogenic

[a]For most of these chemicals, the results of long-term studies were readily available, or preliminary findings could have been obtained. These results were not consulted during the prediction process.

relationships do exist for single experiments or individual chemicals. Yet, when generic statements are proposed, using induced cell proliferation as a recent and continuing example of mechanistic-based carcinogenesis, experimental findings fail to support the often global notions made as a result (*9,13,35,36*).

Thus one often may be misled to predict carcinogenesis based on observations from shorter term studies that purportedly are associated with carcinogenesis. For example, hyaline droplet formation (re $\alpha_{2\mu}$ globulin), induced or enhanced cell turnover, kidney or urinary bladder stones, goiter, nephropathy, increased liver weights, tissue necrosis and adaptive regeneration may be placed in this category of poorly generalized predictors. Associations with these connectors are typically identified only after the long-term studies are completed; yet little is reported about the myriad times no correlations could be made for these same observations. Another example is organ specific DNA adduct formation. This biomarker was highly valued as a predictor of carcinogenesis until more numerous findings showed organs exhibiting cancer but no adduct formation, and organs showing DNA adducts but no cancer. One of the values, of course, is as a biomarker measure of exposure, which may prove to be the purpose of induced hyaline droplets or $\alpha_{2\mu}$-globulin.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) may be the one chemical that caused the largest avalanche of mechanistic research in a continuing effort to discover and explain why or how this nonmutagenic (*37*) carcinogen was positive in the carcinogenesis bioassay (*38–40*). Clearly much of this research may have been stimulated by the Seveso accident in 1976 (*41–43*). Decades later, however, we remain uncertain about the mechanism(s) of carcinogenesis of TCDD (*44,45*), or for any other chemical carcinogen.

Even when we and others (*46*) decided to test benzene, we were challenged because everyone "knew already" that benzene caused leukemia in humans, and previous *in vivo* tests in animals failed to show carcinogenic activity (*47,48*); thus the following question was raised: why test this

chemical again and waste further time, more money, and scarce facilities on a "known" negative? Yet the major thrust was not only to determine if indeed benzene would be carcinogenic in more modern and adequately designed protocols, but also if this clastogenic human carcinogen would be detected in our rodent models and hence would tend to further validate these models. Despite the many previous negative (and largely inadequate) studies on benzene, this chemical was considered "quite" carcinogenic in our test systems (*49,50*). Predictions at the time thus centered on a negative outcome.

Another example of a known human carcinogen, and a so far marginal-evidence carcinogen in rodents is arsenic. After many years of attempting to have this metal tested in the NTP protocol, the bioassay is now being designed under the rubric of mechanistic studies. The point here is that some still insist that the bioassay is not relevant to humans simply because this single human carcinogen has not been shown to be a potent or convincing carcinogen in rodents. Few mention that the studies done till now have been woefully inadequate (*51,52*). Nonetheless, we wonder, if inorganic arsenic were on this exercise list of 30 chemicals, how many of the participants would place it in the carcinogen category.

Another interesting and controversial example is trichloroethylene (TCE), a high-volume solvent and degreaser once used to decaffeinate coffee. When the first test results were made available in 1976, the studies caused considerable debate because some charged that the TCE- induced carcinogenicity was due to or influenced by a small amount of epichlorohydrin used as a stabilizer in the commercial product. First, TCE was not predicted to cause cancer in laboratory animals; and second, the pattern of tumors was different from that seen with epichlorohydrin alone. Nonetheless, TCE (without epichlorohydrin) was retested, and shown again to be carcinogenic. Now we learn that TCE has been shown to cause kidney cancer in humans (*53*), a site common to that seen previously in animals. This was the first in a long series of short-chain halogenated aliphatic hydrocarbon solvents shown to consistently cause tumors in laboratory animals. Without knowing all this information, we suspect TCE would be predicted not to be carcinogenic. Ironically, TCE was replaced with another short-chain halogenated aliphatic hydrocarbon, methylene chloride, in decaffeinated coffee (*54*).

On the other hand, and using what we know already about empirical or qualitative chemical carcinogenesis, we have more predictive conviction on certain chemicals or classes of chemicals. For instance, we believe no other benzidine-based dyes, nitrosamines, aminoanthraquinone pigments, phenylenediamines, anilines, or benzo[*x*]pyrenes, among others, need be tested for carcinogenicity. That is, for these groups or classes of chemicals, should we not assume with some degree of scientific confidence that such chemicals would be carcinogenic, and not bother with confirmatory long-term studies? Of course exceptions do and will exist, but perhaps the producers, or sellers of a new aminoanthraquinone dye, for example, should be obligated to prove noncarcinogenicity before marketing, rather than force others to prove carcinogenic activity.

Even in these situations one must be receptive to contradictions. We felt almost obligated to predict that emodin (an anthraquinone) would not possess any carcinogenic activity or would simply give a marginal or equivocal tumor response; we reached this conclusion because emodin, although mutagenic to *Salmonella,* is 1, 3, 8-trihydroxy-6-methyl anthraquinone that ostensibly would be easily and rapidly metabolized, conjugated, and excreted. The more we thought about this, and with the awareness that 1-hydroxyanthraquinone; 1,8-dihydroxyanthraquinone; and 1,2,4-trihydroxyanthraquinone are each carcinogenic, often with different target organs, we elected to place emodin in the anthraquinone-carcinogen category. Our original predisposition was diverted with information that we thought was more important and relevant.

Conversely, from the beginning, we thought that the parent to this class of chemicals, anthraquinone, would be carcinogenic based largely on prechronic toxic and proliferative effects (which mimicked substituted anthraquinones shown to be carcinogenic), positive mutagenicity findings, and *in vivo* micronuclei induction. However, we decidedly placed more importance on the likelihood of rapid metabolism and elimination via 2-hydroxyanthraquinone rather than on the shorter term toxicity test results. Further, we hypothesized that the observed carcinogenic activity of the 1- and 8-hydroxyanthraquinones may be due to formation of a pyrene-type structure via hydrogen bonding between the hydroxyl and carbonyl functions. Formation of this structural moiety is not possible for 2-hydroxyanthraquinone, and thus we predicted that anthraquinone would not be carcinogenic. Whether this turns out as we predict awaits the real data.

Scopolamine, a naturally occurring tropane alkaloidal anticholinergic chemical, proved moderately difficult to predict. It was virtually devoid of mutagenic activity in a battery of *in vitro* (except weakly positive for chromosomal aberrations in CHO cells with activation) and *in vivo* assays (the mouse peripheral blood micronucleus test). It is rapidly absorbed (bioavailability = 20–30%), and excreted ($t_{1/2}$ = 3 hrs). The CSWG predicted a carcinogenic response because scopolamine contains an aliphatic epoxide moiety and potential for nitrosation of the amino group. Yet, none of the short-term tests revealed mutagenic or alkylating activity. No toxicology was observed in 16-day or 14-week studies. Also, it is possible that the epoxide may not be reactive due to its hindered location. Nonetheless, because of this structural alert we placed this agent in the marginal or equivocal, borderline-to-none, category.

At present, and for a relatively long history, the most certain method by which to identify potential human carcinogens comes from exposing laboratory animals to particular exposure circumstances similar to those of humans—chemical, mixtures of chemicals, occupations, work places, lifestyles, personal habits, environmental conditions, and combinations of these—for long periods. Perhaps some day, human expert–computer–machine learning interactive methods will become more adept, proficient, and accurate at utilizing minimal or no available information to predict which exposures will be carcinogenic, and which of those will then most likely represent the greatest carcinogenic risks to exposed populations. These and other *in vitro* and *in vivo* alternatives have been searched for over the decades, with some, albeit limited, success for complementing the bioassay. None, however, have been considered successful enough, in our view, to replace or in most cases even reduce reliance on the long-term bioassay. Some methods have striven to simply shorten the exposure times by developing *in vivo* assays whereby select initiators have been used (*55*).

Despite these noble attempts, the current bioassay, with its recognized faults and shortcomings, has survived both valued and misguided criticisms to discredit its worthiness and reliability as well as balanced scientific and public health-based

efforts to replace, reduce, refine, or eliminate the use of and dependence on it.

We hope that new methods will be developed that can eventually replace the current bioassay. These methods should be quicker and less expensive than the current bioassays, and should use few or no animals, and be fully predictive of effects in humans. Meanwhile, and to best protect public health, exposure circumstances should be evaluated at an increasing pace using *in vivo* long-term bioassays.

REFERENCES

1. Tomatis L, Aitio A, Wilbourn J, Shuker L. Human carcinogens so far identified. Jpn J Cancer Res 80:795–807 (1989).
2. Huff JE. Chemicals causally associated with cancers in humans and in laboratory animals: a perfect concordance. In: Carcinogenesis (Waalkes MP, Ward JM, eds). New York:Raven Press, 1994;25–37.
3. Huff JE. Design strategies, results, and evaluations of long-term chemical carcinogenesis studies. Scand J Work Environ Health 18 (Suppl 1):31–37 (1992).
4. Huff JE, Haseman JK, Rall DP. Scientific concepts, value, and significance of chemical carcinogenesis studies. Annu Rev Pharmacol Toxicol 31:621–652 (1991).
5. Weisburger EK. Might it be a carcinogen? Chem Technol 17:422–424 (1987).
6. Fung VA, Huff JE, Weisburger E, Hoel DG. Predictive strategies for selecting 379 NCI/NTP chemicals evaluated for carcinogenic potential: scientific and public health impact. Fundam Appl Toxicol 20:413–436 (1993).
7. Woo YT, Lai DY, Argus MF, Arcos JC. Development of structure–activity relationship rules for predicting carcinogenic potential of chemicals. Toxicol Lett 79:219–228 (1995).
8. Boyd JA, Barrett JC. Genetic and cellular basis of multistep carcinogenesis. Pharmacol Ther 46:469–486 (1990).
9. Huff JE. Mechanisms, chemical carcinogenesis, and risk assessment: cell proliferation and cancer. Am J Ind Med 27:293–300 (1995).
10. Vainio H, Magee PN, McGregor DB, McMichael AJ, eds. Mechanisms of Carcinogenesis in Risk Identification. IARC Scientific Publications 116. Lyon:International Agency for Research on Cancer, 1992;1–616.
11. Barrett JC. Mechanisms for species differences in receptor-mediated carcinogensis. Mutat Res 333:189–202 (1995).
12. Huff JE, Boyd JA, Barrett JC, eds. Cellular and Molecular Mechanisms of Hormonal Carcinogenesis: Environmental Influences. New York:Wiley-Liss, 1996.
13. Farber E. Cell proliferation as a major risk factor for cancer: a concept of doubtful validity. Cancer Res 55:3759–3762 (1995).
14. Huff JE. Issues and controversies surrounding qualitative strategies for identifying and forecasting cancer causing agents in the human environment. Pharmacol Toxicol 72 (Suppl 1):12–27 (1993).
15. Lucier GW. Mechanism-based toxicology in cancer risk assessment: implications for research, regulation, and legislation. Environ Health Perspect 104:84–88 (1996).
16. Wachsman JT, Bristol DW, Spaulding J, Shelby M, Tennant RW. Predicting chemical carcinogenesis in rodents. Environ Health Perspect 101:444–445 (1993).
17. Hileman B. "Expert intuition" tops in test of carcinogenicity prediction. Chem Eng News 21 June 1993;35–36.
18. Bristol DW. Summary and recommendations for Session B: activity classification and structure–activity relationship modeling for human health risk assessment of toxic substances. Toxicol Lett 79:265–280 (1995).
19. Fung VA, Barrett JC, Huff JE. The carcinogenesis bioassay in perspective: application in identifying human cancer hazards. Environ Health Perspect 103:680–683 (1995).
20. Chhabra RS, Huff JE, Schwetz BS, Selkirk J. An overview of prechronic and chronic toxicity/carcinogenicity experimental study designs and criteria used by the National Toxicology Program. Environ Health Perspect 86:313–321 (1990).
21. Huff JE, Hoel DG. Perspective and overview of the concepts and value of hazard identification as the initial phase of risk assessment for cancer and human health. Scand J Work Environ Health 18 (Suppl. 1):83–89 (1992).
22. Rall DP, Hogan MD, Huff JE, Schwetz BA, Tennant TW. Alternatives to using human experience in assessing health risks. Annu Rev Public Health 8:355–385 (1987).
23. NTP. Carcinogenesis Bioassay of Di(2-ethylhexyl)phthalate (CAS No. 117-81-7) in F344 Rats and B6C3F1 Mice (Feed Studies). NTP Technical Report Series TR No 217. Research Triangle Park, NC:National Toxicology Program, 1982;1–127.
24. Kluwe WM, Haseman JK, Huff JE. The carcinogenicity of di(2-ethylhexyl)phthalate (DEHP) in perspective. J Toxicol Environ Health 12:159–169 (1983).
25. Huff JE, Kluwe WM. Phthalate esters carcinogenicity in F344 rats & B6C3F1 mice. In: Industrial Hazards of Plastics and Synthetic Elastomers (Jarvisalo J, Pfaffli P, Vainio H, eds). New York:AR Liss, 1984;137–154.
26. NTP. Toxicology and Carcinogenesis Studies of *d*-Limonene (CAS No. 5989-27-5) in F344 Rats and B6C3F$_1$ Mice (Gavage Studies). NTP Technical Report Series TR No 347. Research Triangle Park, NC:National Toxicology Program, 1990;1–165.
27. Barrett JC, Huff JE. Cellular and molecular mechanisms of chemically induced renal carcinogenesis. In: Nephrotoxicity. Mechanisms, Early Diagnosis, and Therapeutic Management (Bach PH, Gregg NJ, Wiks MF, Delacruz L, eds). New York:Marcel Dekker, 1991;287–306. Revised and updated: Renal Fail 13:211–225 (1991).
28. Huff JE. Chemical toxicity and chemical carcinogenesis. Is there a causal connection? A comparative morphological evaluation of 1500 experiments. In: Mechanisms of Carcinogenesis in Risk Identification. IARC Scientific Publications 116 (Vainio H, Magee P, McGregor D, McMichael A, eds). Lyon:International Agency for Research on Cancer, 1992;437–475.
29. Melnick RL. An alternative hypothesis on the role of chemically induced protein droplet ($\alpha_{2\mu}$ globulin) nephropathy in renal carcinogenesis. Reg Toxicol Pharmacol 16:111–125 (1992).
30. Borghoff SJ, Lehman-Mckeeman LD, Short BG, Hard GC, Swenberg JA. Critique of R. Melnick's. "An alternative hypothesis on the role of chemically induced protein droplet ($\alpha_{2\mu}$ globulin) nephropathy in renal carcinogenesis." Regul Toxicol Pharmacol 18:357–364 (1993).
31. Melnick RL. Critique does not validate assumptions in the model on $\alpha_{2\mu}$ globulin and renal carcinogenesis. Regul Toxicol Pharmacol 18:365–368 (1993).
32. Melnick RL, Kohn M, Portier CJ. Implications of risk assessment of suggested nongenotoxic mechanisms of chemical carcinogenesis. Environ Health Perspect 104 (Supp 1):123–134 (1996).
33. Tennant RW, Elwell MR, Spalding J, Griesemer RA. Evidence that toxic injury is not always associated with induction of chemical carcinogenesis. Mol Carcinog 4:420–440 (1991).
34. Huff JE. Absence of morphologic correlation between chemical toxicity and chemical carcinogenesis. Environ Health Perspect 101 (Suppl. 5):45–54 (1993).
35. Melnick RL, Huff JE. Liver carcinogenesis is not a predicted outcome of chemically induced hepatocyte proliferation. Toxicol Ind Health 9:415–438 (1993).
36. Melnick RL, Huff JE, Barrett JC, Maronpot RR, Lucier G, Portier CJ. Cell proliferation and chemical carcinogenesis: a symposuim overview. Mol Carcinog 7:135–138 (1993).

37. Wassom JS, Huff JE, Loprieno N. A review of the genetic toxicology of chlorinated dibenzo-*p*-dioxins. Mutat Res 47:141–160 (1977).
38. NTP. Carcinogenesis Bioassay of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (CAS No 1746-01-6) in Osborne-Mendel Rats and B6C3F_1 Mice (Gavage Study). NTP Technical Report Series TR No 209. Research Triangle Park, NC:National Toxicology Program, 1982;1–195.
39. Zeise L, Huff JE, Salmon AG, Hooper NK. Human risks from 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and hexachlorodibenzo-*p*-dioxins. Adv Mod Environ Toxicol 17:293–342 (1990).
40. Huff JE. Dioxins and mammalian carcinogenesis. In: Dioxins and Health (Schecter A, ed). New York:Plenum Press, 1994;389–407.
41. Huff JE, Wassom JS. Chlorinated dibenzodioxins and dibenzofurans. An annotated literature collection 1934–1973. Environ Health Perspect 5:283–312 (1973).
42. Huff JE, Wassom JS. Health hazards from chemical impurities: chlorinated dibenzodioxins and dibenzofurans. Int J Environ Studies 6:13–17 (1974).
43. Huff JE, Moore J, Saracci R, Tomatis L. Long-term hazards of poly-chlorinated dibenzodioxins and polychlorinated dibenzofurans. Environ Health Perspect 36:221–240 (1980).
44. Huff JE. 2,3,7,8-TCDD: a potent & complete carcinogen in experimental animals. Chemosphere 25:173–176 (1992).
45. Huff JE, Lucier GW, Tritscher AM. Carcinogenicity of TCDD: experimental, epidemiologic, and mechanistic evidence. Annu Rev Pharmacol Toxicol 34:343–372 (1994).
46. Maltoni C, Ciliberti A, Cotti G, Contri B, Belpoggi F. Benzene, an experimental multipotential carcinogen: results of the long-term bioassays performed at the Bologna Institute of Oncology. Environ Health Perspect 82:109–124 (1989).
47. IARC. Benzene. In: Some Industrial Chemicals and Dyestuffs. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Vol 29. Lyon:International Agency for Research on Cancer, 1982;93–148; 391–398.
48. IARC. Benzene. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluation of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42 (Suppl 7). Lyon:International Agency for Research on Cancer, 1987;120–122.
49. Huff JE, Haseman JK, DeMarini DM, Eustis SL, Maronpot RR, Peters AC, Persing RL, Chrisp CE, Jacobs AC. Multiple-site carcinogenicity of benzene in Fischer rats and B6C3F_1 mice. Environ Health Perspect 82:125–163 (1989).
50. Huff JE. Applicability to humans of rodent-specific sites of chemical carcinogenicity: tumors of the forestomach and of the harderian, preputial, and zymbal glands induced by benzene. J Occup Med Toxicol 1:109–141 (1992).
51. IARC. Arsenic. In: Some Metals and Metallic Compounds. IARC Monograph on the Evaluation of Carcinogenic Risks to Humans, Vol 23. Lyon:International Agency for Research on Cancer, 1980;39–141.
52. IARC. Arsenic. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluation of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42 (Suppl 7). Lyon:International Agency for Research on Cancer, 1987;100–106.
53. Henschler D, Vamakas S, Lammert M, Dekant W, Kraus B, Thomas B, Ulm K. Increased incidence of renal cell tumours in a cohort of cardboard workers exposed to trichloroethylene. Arch Toxicol 70: 131–133 (1995).
54. Huff JE, Bucher J, Barrett JC. Methylene chloride. Science 272:1083–1084 (1996).
55. Ito N, Hasegawa R, Imaida K, Hirose M Shirai T. Medium-term liver and multi-organ carcinogenesis bioassays for carcinogens and chemopreventive agents. Exp Toxicol Pathol 48:113–119 (1996).